A STUDY OF HYPOTHESES ON THE ROLE OF ELECTRICAL FORCES IN MITOSIS

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ELECTRICAL FORCES IN MITOSIS

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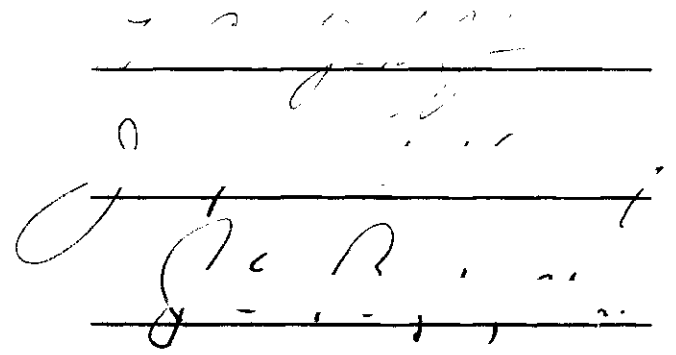

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INTRODUCTION

During the past fifty years a number of hypotheses have been advanced concerning the part played by electrical forces in the process of mitosis. A superficial examination of the literature indicated that none of these postulates presents an adequate explanation of the observed phenomena.

The purpose of this study was to ascertain the exact scope of these hypotheses and to evaluate them in terms of the experimental facts as they are now known. It was hoped that such a review and evaluation would indicate the most desirable course for future study and experimentation on this problem.

In formulating the results of this study, the process of mitosis has been reviewed, and both typical and atypical phenomena have been considered. Illustrations of the phases of mitosis have been made to facilitate comparison with results obtained by experimentation with physical systems.

The various hypotheses concerning the role of electrical forces in mitosis have been reviewed in the order in which they were proposed. Experimental findings in support or refutation of a particular concept have been included with the discussion of the hypothesis. In conclusion, the hypotheses and the experimental findings have been compared and eval-

uated, and possible fields of future research and experimentation have been indicated.

In this study, the term mitosis has been used to include all nuclear divisions that involve the equal partition of the chromosomes to the daughter nuclei. The term meiotic division has been used to designate specifically those mitoses which include a reduction in the number of chromosomes in the nucleus.

THE PROCESS OF MITOSIS

The course of a typical mitosis may be divided arbitrarily into five stages: interphase, prophase, metaphase, anaphase, and telophase. This division is simply one of convenience, and there is no sharp demarkation between phases.

Interphase. The period in the life history of cells between active divisions is known as interphase and is the state in which the cells remain for the greatest time. The appearance of a typical cell fixed and stained during interphase is shown in Figure 1. The nucleus is generally spheroidal with the chromatin distributed in the form of a diffuse network. One or more dark bodies, the nucleoli, are usually present within the nucleus. Lying just outside the nuclear membrane is a small ovoid structure, the centrosome, which appears quite clear except

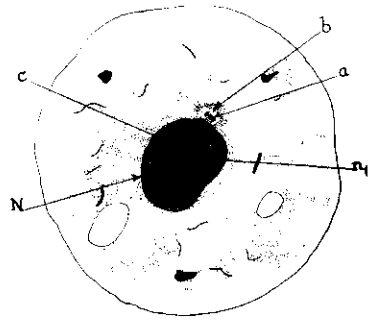

Figure 1. Interphase Cell
a, centriole; b, centrosome,
c, chromatin; n, nucleolus;
N, nucleus.

for two deeply staining granules, the centrioles. Around the centrosome, a system of radiating fibers or rays is visible. The structure formed by the centrosome, the centrioles, and the rays is called an aster.

Prophase. In early prophase, Figure 2, the chromatin network changes into a coiled thread-like form, the spireme. This may be one continuous thread, but is generally broken into a number of shorter ones. The spireme may result from a simple gradual transformation of the network or from a condensation of the network into several globular masses, each of which is later converted into a thread. The spireme itself then shortens and thickens, producing first a coarse spireme, Figure 3, and then several discrete bodies, the chromosomes.

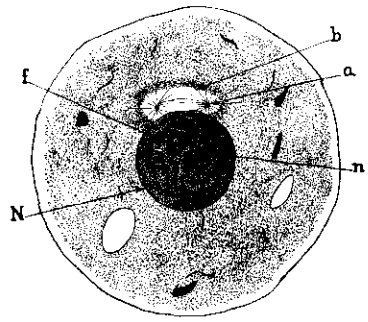

Figure 2. Early Prophase
f, fine spireme.

At some time during the conversion of the chromatin network into the chromosomes, the threads split longitudinally so that the chromosomes are formed in identical pairs. Most authors, basing their conclusions on visual observation, believe this doubling occurs during the fine spireme state. Some, however, contend it does not occur until the coarse spireme stage, and others place it as early as the anaphase of the preceeding division. The cause of this wide discrepancy in results is probably the fact that the fine spireme is, in most cases, near the limit of resolution of the microscope, so that it is very difficult to detect the doubling if it is present. Mather seems to have settled this question,

at least in Tradescantia and Eremurus, by the use of X-rays.[1] It is known that X-rays will produce breaks in the spireme which become apparent in later phases, since chromosomes of abnormal length are formed. By determining the earliest time at which X-rays can produce this abnormality in only one of a pair of chromosomes, Mather has shown that the doubling occurs in late interphase.

During this transformation of the chromatin network, the rays of the aster increase in number and in length. At about the time of the coarse spireme stage, shown in Figure 3, the centrosome divides, forming two asters with one centriole in each. These asters separate and begin to move to opposite ends of the nucleus. Some of the rays between the separating asters seem to fuse to form continuous fibers extending from one aster to the other. These continuous fibers give rise to a spindle between the asters. Some distance from the spindle axis, fibers may be observed which cross at a definite angle. This fact has been a major objection against electrical forces as the basic cause of mitosis. A readjustment of the rays, during which they curve so as to form additional continuous fibers eventually causes the disappearance of this crossing. The spindle is very incomplete

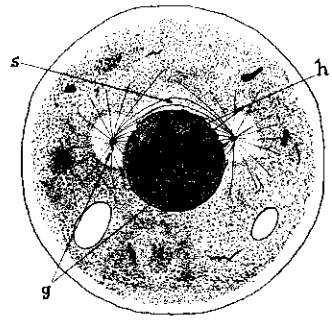

Figure 3. Mid-prophase g, asters; h, coarse spireme; s, spindle.

[1] Mather, K., "The Experimental Determination of the Time of Chromosome Doubling," Proceedings of the Royal Society of London, Series B, 124:97-106, 1937-38.

at this stage because the nucleus intercepts part of the fibers. At the points of contact of the fibers and the nuclear membrane, the latter frequently appears to be pushed inward by the fibers. Shortly before the asters reach opposite sides of the nucleus, about the time of formation of definite chromosomes, the nuclear membrane begins to disintegrate, apparently at the points of contact with the fibers. This late prophase condition is illustrated in Figure 4.

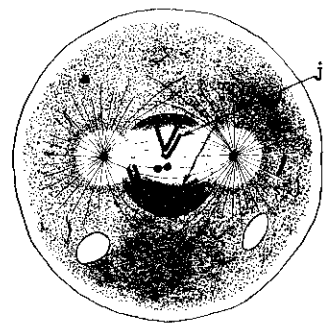

Figure 4. Late Prophase
j, chromosomes.

Metaphase. During early metaphase the astral fibers increase in length and number so that the spindle is completed, and the rays not taking part in the spindle formation extend nearly to the outer membrane of the cell. The chromosomes migrate from the positions in which they were formed to the equatorial region of

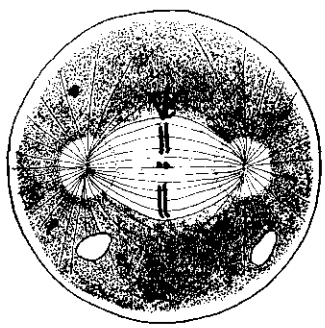

Figure 5. Full Metaphase
Side View

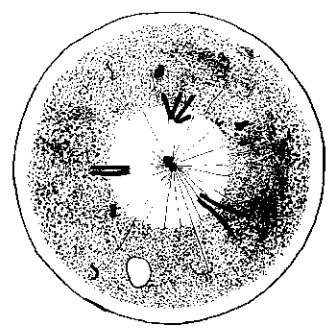

Figure 6. Full Metaphase
Polar View

the spindle. At full metaphase, the chromosomes lie in the equatorial plane in positions such that the members of each pair are on opposite

sides of this plane. Figures 5 and 6 show the side view and polar view, respectively, of the cell at full metaphase. There seems to be a tendency for the smaller chromosomes to take positions near the center of the metaphase group, and for those of equal size to lie side by side. Some time during metaphase each chromosome becomes attached to a fiber from the nearest aster. This attachment takes place at only one point, the kinetochore, in each chromosome. At about this time, the centriole in each of the asters divides, but the two new centrioles thus formed remain inside the old centrosome, which does not divide. Metaphase is a relatively stable configuration in which the mitotic figure may remain for some time.

Anaphase. During anaphase the chromosome pairs separate and move along the path of the spindle fibers to the poles. The separation of the pairs begins at the kinetochore and proceeds as if the driving force were acting only at that point, the remainder of the chromosome being simply dragged along. This action may produce some startling changes in the shape of the chromosomes. A chromosome having a rod shape during metaphase will assume a V shape during the anaphase movement if its kinetochore is centrally located, or a J shape if the kinetochore is subterminal in position. Figure 7 shows the appearance of the mitotic figure in mid-anaphase. In some cases, at least, the anaphase movement of the chromosomes seems to be a two step process with a considerable pause

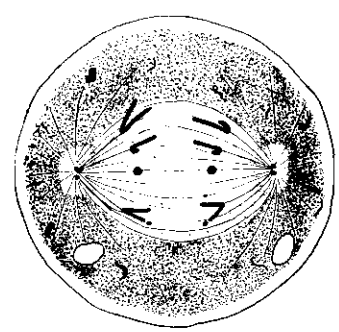

Figure 7. Mid-anaphase

between the two motions.[2]

Telophase. The chromosomes at the spindle poles disintegrate into a diffuse chromatin network in telophase, and a new nuclear membrane is formed around each mass of chromatin. Usually the cytoplasm also divides by constricting in the equatorial plane of the disintegrating spindle, Figure 8. The aster decreases in size and assumes a position just outside the new nuclear membrane. Thus the daughter cells assume the interphase condition, and the mitotic process is completed.

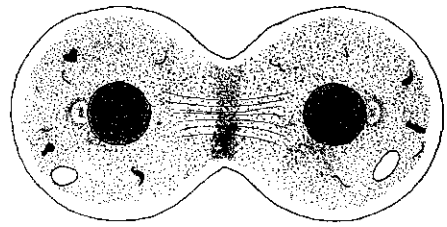

Figure 8. Telophase

The nucleolus in mitosis. The fate of the nucleolus in mitosis is not definitely known in most cases and seems to vary in different types of cells. Thus Wilson[3] points out that in some instances the nucleolus persists with practically no change in form during prophase but rapidly decreases in size, disintegrates, and disappears at the later stages, while in other cases it is cast out bodily after the completion of the mitotic figure. Zirkle[4] found in Zea mays that part of the nucleolar material flows into the spireme during prophase as though the spireme

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[2]Ris, Hans, "A Quantitative Study of the Anaphase Movements in the Aphid Tamalia," Biological Bulletin, 85:164-78, 1943.

[3]Wilson, E. B., The Cell in Development and Heredity, pp. 141-42.

[4]Zirkle, Conway, "Nucleolus in Root Tip Mitosis in Zea mays," Botanical Gazette, 86:402-20, 1928.

were a collapsed tube. The remainder of the nucleolus splits and migrates to the poles where it disintegrates and passes out into the cytoplasm. On the other hand, in Spirogyra, the chromosomes form in early prophase while the nucleolus is still intact. About mid-prophase, the nucleolus breaks down to form a granular homogeneous substance in which the remainder of the mitotic process takes place.[5] Gates[6] confirmed these observations on Spirogyra but found in Ciliata that the nucleoli remain as elongated structures surrounding portions of certain chromosomes.

The reality of spindle fibers. The astral rays are clearly visible in both living and fixed materials. The fibers of the spindle, however, have been seen in living materials only in a very few cases. In normal living cells only two materials have been reported in which the spindle fibers are clearly visible. Even these represent somewhat specialized conditions. Cooper[7] was able to see the spindle fibers clearly in the blastomeres of the mite, but in these cells the chromosomes are contained in separate karyomeres which persist until late in the cycle. In certain protozoan flagellates, Cleveland[8] was able to see both continuous and chromosomal fibers in the spindles. In the flagellates the

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[5]Doraiswami, S., "Nuclear Division in Spirogyra," Journal of the Indian Botanical Society, 21:16-36, 1941.

[6]Gates, R. R., "Nucleoli and Related Nuclear Structures," Botanical Review, 8:337-409, 1942.

[7]Cooper, K. W., "Visibility of the Primary Spindle Fibers and the Course of Mitosis in the Living Blastomeres of the Mite Pediculopsis graminum Reut," Proceedings of the National Academy of Science, 27: 480-84, 1941.

[8]Cleveland, L. R., "The Centriole and its Role in Mitosis as Seen in Living Cells," Science, 81:598, 1935.

nuclear membrane remains intact throughout the mitotic process. Schrader[9] cites many works of an indirect character which clearly show the reality of the longitudinal structure of the spindle and prove it is not a staining or fixation artifact. Whether this structure is actually made up of fibers in the usual sense of the word is not known. Each attempt to explain the forces active in mitosis demands its own assumptions regarding the exact nature of the spindle fibers, and experimental indications can be assembled for each hypothesis.[10]

Atypical mitoses. Mitoses which show major deviations from the process outlined above are very numerous. The most important of these for the present study are those which are not bipolar. Many mitoses occur with the formation of only one aster. In most of these, the daughter chromosomes do not separate but remain close together and are all included in one nucleus.[11] In Sciara, the first spermatocyte division is regularly monocentric and the chromosomes are separated, half of them going to the pole while the others move to the cell periphery.[12] In contrast to these, numerous instances of multipolar mitoses are reported.[13] Tripolar mitoses with spindles between each aster and tetrapolar figures with six spindles have frequently been used to discredit any hypothesis

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[9]Schrader, Franz, Mitosis, pp. 9-16.

[10]Ibid., pp. 39-44.

[11]Wilson, E. B., op. cit., pp. 168-72.

[12]Metz, C. W., "Monocentric Mitosis with Segregation of Chromosomes in Sciara and its Bearing on the Mechanism of Mitosis," Biological Bulletin, 64:333-47, 1933.

[13]Wilson, E. B., op. cit., pp. 172-73.

involving a bipolar force field.[14]

Another variation from the typical mitosis which is frequently found is the anastral mitosis. The extreme form of this is the complete absence of asters at the ends of the spindle. With this condition, the spindle fibers may diverge at the end instead of converging to a pole. The rays may also converge nearly as much as in the astral figures, the only difference then being the lack of the centrosome and the astral rays. In other variations, the centrosome may be present without any centriole. From the numerous variations in the form of the aster and the centriole, it seems quite possible that anastral mitoses simply represent configurations in which the polar substance is diffuse rather than concentrated in a polar granule.[15]

THE HYPOTHESES

The striking superficial resemblance of the mitotic figure to the configuration assumed by iron filings in a bipolar magnetic field was emphasized by Fol in the early 1870's. Ziegler[16] was one of the first to attempt to extend this resemblance. Using various combinations of horseshoe magnets placed beneath a thin wax plate, he demonstrated that iron filings dusted on the plate will assume configurations very similar to those seen in some polycentric mitoses. Ziegler, however, did not make

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[14] Ibid., p. 188.

[15] Schrader, F., op. cit., p. 17.

[16] Ziegler, H. E., "Untersuchungen über die Zelltheilung," Verhandlungen der Deutschen Zoologischen Gesellschaft, 5:62-83, 1895.

any effort to carry the analogy further.

Gallardo's hypothesis. The first attempt to show that the forces active in mitosis are of a nature similar to electric and magnetic forces was made by Gallardo in 1896.[17] He did not specify the exact nature of this force, but spoke of it only as the karyokinetic force. He assumed that the centrosomes became polarized with opposite signs and, at metaphase, attracted the daughter chromosomes nearest to them with sufficient force to bring about their anaphase movement.

On these assumptions, Gallardo explained the process of mitosis in the following manner. Initially, the cytoplasm becomes polarized in two regions on opposite sides of the cell. In some unexplained manner, the prophase movements of the centrosomes are brought about, and they acquire, during this movement, the polarization of the cytoplasmic region into which they move. The spindle formed between the centrosomes increases in size during prophase due to the increased polarization between these centers. By full metaphase, the centrosome polarization has reached a maximum which is sufficient to pull apart the halves of the metaphase chromosomes, which must also be polarized, and to draw them to the poles. When the chromosomes and centrosome combine to form the late telophase nucleus, the net polarization is reduced to zero and the spindle therefore ceases to exist.

To demonstrate further the similarity of the mitotic field to a polarized force field, Gallardo produced three-dimensional models using

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[17]Gallardo, A., "Essai d'Interprétation des Figures Karyokinétiques," Anales Museo Nacional de Buenos Aires, 5:11-22, 1896.

electrostatic forces. Into a narrow glass trough containing a suspension of quinine sulfate in turpentine he introduced two small spheres connected through conducting threads to the poles of an electrostatic machine. When the field was applied, the crystals oriented themselves along the lines of force of the field and thus produced figures resembling the mitotic spindle and asters. The spindle formed gave the appearance of a group of fibers, and these could be displaced or distorted with a glass rod without losing their continuity. Fibers displaced by the rod returned to their original positions when it was removed. By introducing a grounded wire, Gallardo was able to produce triastral figures.

One of the most serious objections to Gallardo's hypothesis was his inability to explain the prophase movements of the centrosomes and the relative stability of the metaphase plate. In any hypothesis involving inverse square attractive forces, any slight disturbance of the metaphase plate which placed the chromosomes nearer one pole should result in their immediate movement to that pole. Such an effect, however, is not observed.[18] According to Wilson,[19] M. Boverie proved in 1903 that two separate asters not connected by a spindle always move more widely apart than those connected by spindles. This must be interpreted as indicating that asters repel each other.

Recognizing these facts, Gallardo revised his earlier hypothesis, and, assuming the mitotic force to be electrostatic in nature, assigned a

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[18]Lillie, R. S., "The Physiology of Cell Division. I. Experiments on Conditions Determining the Distribution of Chromatic Matter in Mitosis," American Journal of Physiology, 15:46-84, 1905.

[19]Wilson, E. B., op. cit., p. 188.

negative charge to the chromosomes and a positive one to the centrosomes and cytoplasm.[20] On these assumptions, the centrosomes repel each other and therefore move apart. The spindle is formed in the attractive field between the separating positively charged centers and the negatively charged chromosomes. The division of the chromosomes was based on the properties of "supersaturated solutions" assumed to exist in the nucleus. Each granule, or small part of the chromosome, grows by precipitation on it of particles from the solution. These particles, being of colloidal nature, bear a charge which is added to the growing granule. This process continues until the mutual repulsive action of the charges exceeds the surface tension forces holding the chromosome together. Once the chromosomes have divided, the electrostatic repulsion between the daughter halves combines with the attraction of the centrosomes to move the chromosomes to the poles. Though Gallardo made no statement regarding the cause of the doubling of the centrosomes, it seems that the process described for the chromosomes should apply equally well to the centers.

To explain the observed crossing of the astral rays, Gallardo adopted the concept of material chains of force as distinguished from geometrical lines of force. This idea was first introduced by Hartog[21] who pointed out that the pattern shown by the iron filings in a magnetic field is the pattern of the field as modified by the filings and not that

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[20]Gallardo, A., "L'Interprétation Bipolaire de la Division Karyocinétique," Anales Museo Nacional de Buenos Aires, 6:259-276, 1906.

[21]Hartog, Marcus, "The Dual Force of the Dividing Cell. Part I. The Achromatic Spindle Illustrated by Magnetic Chains of Force," Proceedings of the Royal Society of London, Series B, 76:548-67, 1905.

due to the poles alone. Specifically, the iron fillings in the field will produce paths of greater permeability than that of the surrounding medium, and the field intensity along these paths will then be increased. By suspending powdered iron in glycerine in a magnetic field, Hartog modeled the mitotic spindles. He found that these powdered iron spindles could be distorted or moved around bodily without breaking up. A horizontal spindle produced in a vertical cylinder of glycerine persisted for three days while gradually sagging under the influence of gravity. These experiments, as well as those of Gallardo cited above, indicate that the material chains of force in viscid media have a certain mechanical strength which causes them to persist even when they are moved so that they no longer coincide with the geometrical lines of force of the exciting field. In these same experiments with powdered iron in glycerine, Hartog observed definite crossing of the material chains of force produced by the action of the magnetic field.[22] Unless the validity of Hartog's work is questioned, these experiments seem to answer the objections raised by Wilson[23] and others to any bipolar force hypothesis.

To illustrate the formation of a number of the more common mitotic figures, Gallardo sketched the equipotential surfaces and the lines of force as they would appear in an electric field with positive poles between which are placed negative bodies. Some of these figures are reproduced in Figure 9. From these figures, Gallardo further proposed that the cytoplasmic division results principally from the change in shape of

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[22] Ibid., p. 556.

[23] Wilson, E. B., op. cit., p. 186.

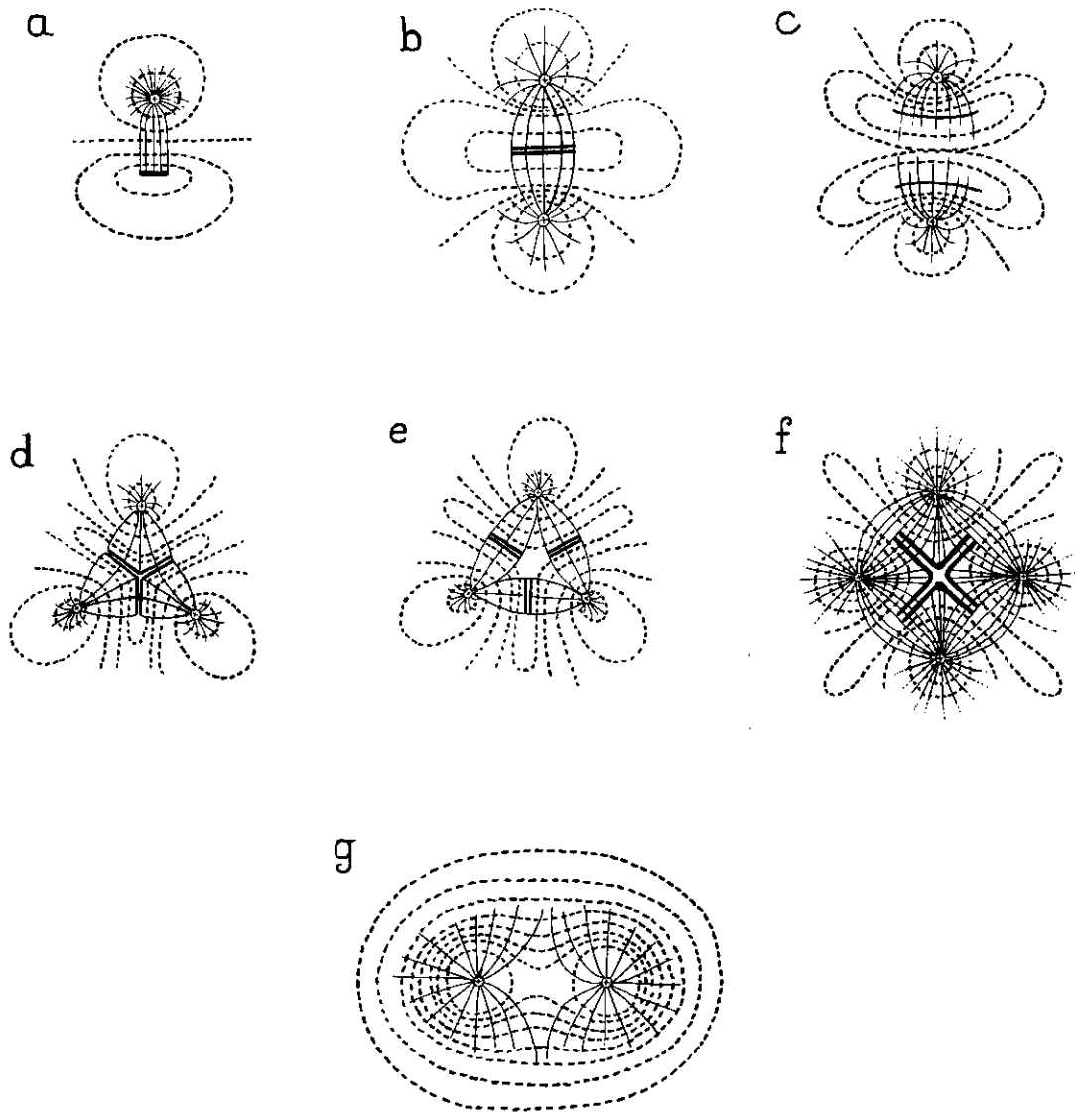

Figure 9. Electrostatic Field Distributions Corresponding to Mitotic Figures

The plus signs indicate the positively charged poles, the solid bars, the negatively charged chromosomes; the solid lines, the lines of force of the field; and the broken lines, the equipotential surfaces. a, monaster; b, normal metaphase; c, normal anaphase; d and e, triasters; f, tetraster with six spindles; g, equipotentials corresponding to the cell membrane.

the equipotential surface corresponding to the cell membrane as the net charge in the two centrosomal regions decreases in late anaphase.

The essential assumptions on which Gallardo's final hypothesis rests may be stated as follows: (1) the centrosomes and cytoplasm are positively charged colloids while the chromosomes are negatively charged; (2) the attractive forces between the chromosomes and the two centrosomes are responsible for the appearance of the spindle and the anaphase movement of the chromosomes; (3) the spindle is formed of material chains of force and does not exactly represent the lines of force of the electrostatic field; and (4) the division of each chromosome is caused by a process of growth, which takes the form of condensation of the colloids of the nucleus, in which the electrostatic charge increases until its force is greater than the surface tension force holding the chromosome together.

Against the third assumption there can be no objection, as the experiments of both Gallardo and Hartog have demonstrated its validity. Perhaps also the fourth proposal could be tentatively accepted, since such growth phenomena are known in colloidal chemistry. On the basis of staining reactions, it can be shown that both the chromosomes and the centrioles must be predominantly acid since both are stained with the basic dyes. In hydrosols, acid particles are negatively charged and basic particles are positively charged. Consequently, the colloids of the chromosomes and of the centrioles must be negatively charged particles. Similarly, since the cytoplasm is stained by acid dyes, its colloids must be predominantly basic, and therefore positively charged. Thus, Gallardo's choice of charges for the chromosomes and the cytoplasm appears to be

logical. The body of the centrosome itself, however, appears clear in most stained preparations. Whether it carries a charge on its outer surface is not known.

The second assumption, however, is open to criticism. First, as has already been noted, a metaphase plate arrangement based on attractive forces between the chromosomes and the poles would be one of relatively unstable equilibrium rather than the stable form observed. Second, on this assumption, chromatin material in some form is necessary for the formation of the spindle, but many cases of quite normal spindles have been reported without the presence of chromatin.[24]

Lillie's hypothesis. Between 1903 and 1916, R. S. Lillie developed a hypothesis which was admittedly only the tentative beginning of an analysis of the complex phenomena of mitosis. The essential features of this proposal are outlined in his paper of 1911.[25] Lillie makes three basic assumptions: (1) that each of the semi-permeable membranes of the cell is the seat of an electrical potential difference due to unequal permeability to anions and cations; (2) that this potential difference will diminish greatly with a marked increase in ionic permeability; (3) that each of the membranes is freely permeable to hydrogen ions.

On the basis of these assumptions, he concluded that the cytoplasmic membrane of the resting cell would exhibit a potential difference positive outside and negative within, while the nuclear membrane would be

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[24] Ibid., p. 176.

[25] Lillie, R. S., "The Physiology of Cell Division. IV. The Action of Salt Solutions Followed by Hypertonic Sea Water on Unfertilized Sea-Urchin Eggs and the Role of Membranes in Mitosis," Journal of Morphology, 22:695-730, 1911.

polarized with the positive surface inside the nucleus. The mitotic process is initiated by a simultaneous and similar change in the ionic permeability of both membranes in regions on opposite sides of the cell. This change results in the depolarization of the membranes. The resulting potential distribution is shown in Figure 10, which is a modification of Lillie's Figure 3.[26] With this distribution, the negative chromosomes are drawn to the equatorial plane, which is the most strongly positive area of the cell.

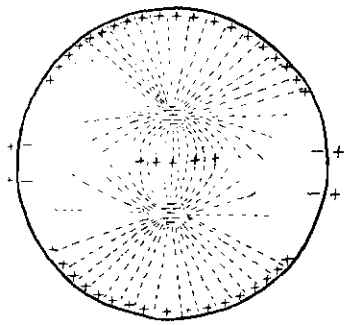

Figure 10. Potential Distribution at Metaphase
Signs indicate relative potentials.

Obviously this hypothesis explains very little of the process of mitosis. It is hard to conceive why the change in permeability should occur in only two localized regions of the membranes. Since the cytoplasm, according to Lillie's own statements, is a poor transmitter of either electrical or diffusion current impulses, the explanation of the simultaneous change in both the cytoplasmic and nuclear membranes is even more difficult. Moreover, assuming these changes, the fact that in many cells the asters originate at the same point in the cytoplasm and then migrate to their metaphase positions, works against Lillie's idea of their mode of formation. The hypothesis also encounters difficulties, as Wilson points out,[27] when an attempt is

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[26] Ibid., p. 727.

[27] Wilson, E. B., op. cit., p. 191.

made to apply it to the formation of large numbers of asters scattered throughout the cytoplasm. In view of these problems, it is doubtful whether Lillie's hypothesis could be accepted as more than a suggestion of a means of explaining the differences in polarization in the resting cell.

Even though Lillie's hypothesis cannot be accepted, many of his experiments give valuable information in regard to the similarity of the mitotic field to that of a bipolar force. From cytological studies it is known that the spireme threads formed in prophase appear to consist of linear series of small bodies which are called chromomeres. In an attempt to simulate this spireme formation, Lillie made an artificial spireme of magnetized needles thrust through small pieces of cork and strung on a fine silk thread.[28] When this assembly was floated on water with the needles in a vertical position and all poles of one sign pointing in the same direction, the filament straightened out due to the mutual repulsions of the magnets. When a large magnet was brought over the floating filament so that the pole nearer the needles attracted them, the thread became convoluted. Under the action of this attractive force, the filament is restricted to a localized area but the individual needles still remain as far from each other as is possible. For a group of filaments whose total length is greater than the circumference of the space in which they must lie, the interaction of these forces results in the coiling of the filaments. Figure 11 shows the form of some of these artificial

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[28]Lillie, R. S., "On the Conditions Determining the Disposition of the Chromatic Filaments and Chromosomes in Mitosis," Biological Bulletin, 8:193-204, 1905.

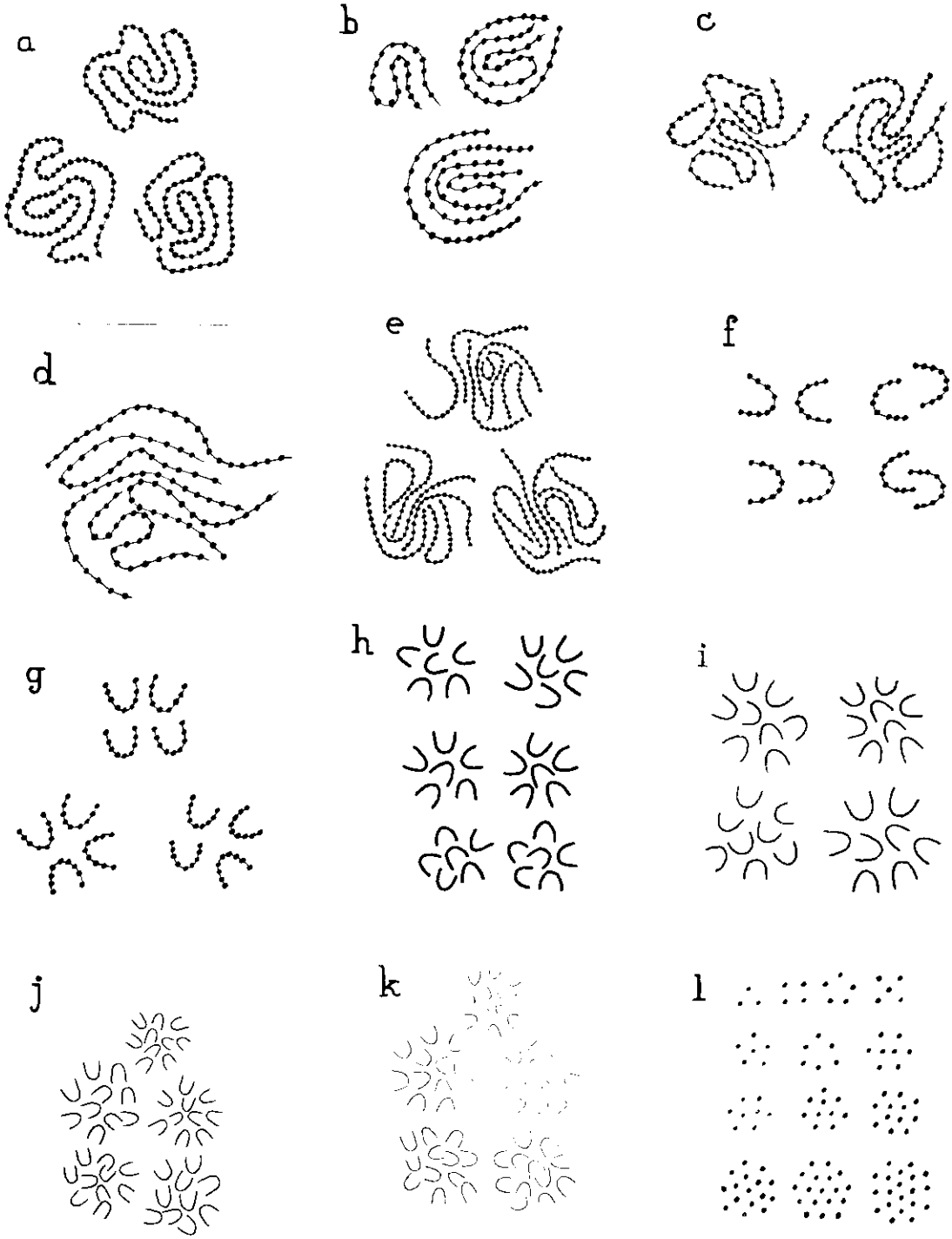

Figure 11. Artificial Spiremes and Metaphase Plates. a-e, configurations assumed by artificial spiremes; f-k, stable arrangements of loop shaped artificial chromosomes made of several magnets strung on wires; l, stable arrangements of single magnets.

spiremes; the dots indicate the positions of the individual needles. Lillie's contention was that the central attractive force supplied by the large magnet is produced in cells by the nuclear membrane or by the centrally acting force of the cytoplasm, while the mutual repulsion of the magnetized needles is analogous to the repulsion between adjacent negatively charged chromomeres.[29]

To produce a magnetic model of the metaphase plate arrangement of the chromosomes, Lillie strung the corks on wires which were bent into a simple open loop shape.[30] When these loops, again floated on water with all needle poles of the same sign uppermost, were subjected to the force of the central attractive magnet, they assumed distributions similar to those of the chromosomes in the metaphase plate. Some of these arrangements are also shown in Figure 11. In these models, the repulsive forces between magnets on each loop are counteracted by the rigidity of the wire. This wire might serve the purpose of the chromosome pellicle; the repulsive forces between units should correspond to the repulsion between negatively charged chromosomes; and the central attractive force supplied by the large magnet would be replaced in the cell by the attraction of the cytoplasm of the equatorial region of the spindle. Obviously these experiments can duplicate the motions of the spireme and chromosomes only in respect to their movement in the plane of the metaphase plate.

Kuwada's hypothesis. Kuwada performed experiments with magnetic models quite similar to some of Lillie's work. To supply the central

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[29] Ibid., p. 199.

[30] Lillie, R. S., "The Physiology of Cell Division. I," op. cit.

attractive force of the central magnet used by the latter, Kuwada[31] employed a current-carrying coil wound around the wooden jar in which his models were floated. Since most cytological evidence indicates that the forces responsible for chromosome movement act only at the kinetochore, and not on the whole body of the chromosome, Kuwada made his models by using single magnetized needles in small corks. To take account of variations in size of the chromosomes, he also used in some experiments larger corks with one magnetized and several unmagnetized needles inserted in them.

For cells with all chromosomes of approximately the same size, the agreement between the metaphase chromosome positions and the positions of the floating magnets is good in fifty to ninety per cent of the observed cases.[32] Figure 12a shows the figure produced by eleven magnets in a stable configuration when all of them are of the same size. This figure corresponds to the arrangement of the chromosomes in the metaphase plate of Cycas revolula.[33] Figure 12b shows an unstable arrangement frequently observed in both the

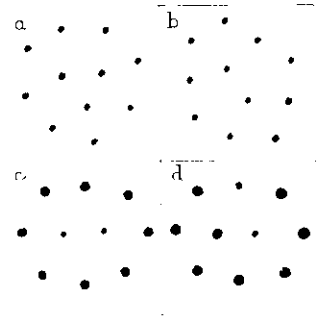

Figure 12. Configurations of Floating Magnets

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[31]Kuwada, Y., "Chromosome Arrangement. I. Model Experiments with Floating Magnets and Some Theoretical Considerations on the Problem," Memoirs of the College of Science, Kyoto Imperial University, 4:199-264, 1929.

[32]Ibid., p. 205

[33]Ibid., p. 206

models and living cells during the transformation to the stable form. The stable configuration is similar to that found by Mayer for eleven magnets of equal size.[34] In his paper Mayer figures the stable arrangements for one to twenty magnets. The configurations produced by magnets of unequal size may not be the same as those with equal magnets. The two most stable arrangements with eight large magnets and two small ones as given by Kuwada are shown in Figure 12, c and d. For ten equal magnets, the most stable form, according to Mayer, has three inner magnets.

The final distribution of the magnets of unequal size depends to a large extent on their distribution at the time the central attractive force is activated. Even with these, however, Kuwada has found good agreement between the stable configurations of the magnets and the chromosome arrangements in metaphase.

On the basis of these experiments, he proposes that the region of the equatorial plane in dividing cells is a positive area to which the negative kinetochores are attracted. The metaphase plate arrangement is due to the combined action of this attraction, the mutual repulsion between the kinetochores of the chromosomes, and the repelling action of the poles on the kinetochores. To account for the anaphase movement of the negative chromosomes to the negative spindle poles, Kuwada postulates a change in sign of the charge on the chromosomes.

This change of sign is demonstrated by the action of the stain, neutral violet extra. This stain colors the cathode of material blue and

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[34]Mayer, A. M., "On the Morphological Laws of the Configurations Formed by Magnets Floating Vertically and Subjected to the Attraction of a Superposed Magnet," London, Edinburgh and Dublin Philosophical Magazine and Journal of Science, 7:98-108, 1879.

the anode red. In prophase it colors the chromosomes blue, while they are stained red at the metaphase plate stage.[35] Zirkle in his work on Zea mays[36] has suggested a similar explanation based on the observation that the electropositive nucleolar material appears to flow into the electronegative spireme.

Kuwada's hypothesis differs little from Lillie's, although he has circumvented the principal objections to the latter simply by ignoring the formation of the centrosomes. He has added the concept of the kinetochore as the point of action of the forces on the chromosome and offered a tentative explanation of the cause of the anaphase movements. The actuality of the change in sign of the chromosome charge remains unconfirmed, and Kuwada does not make it clear whether the kinetochore also shows this change. It seems doubtful that Zirkle's explanation of this alteration in charge could be extended generally since in some cases the nucleolus is known to remain intact until after the completion of the mitotic figure. Even in Zea, the timing of the change is not satisfactory, since Zirkle shows that the change in charge occurs in prophase, while the alteration in chromosome action does not occur until after full metaphase. That the nucleolus and cytoplasm are positively charged, and the chromatin negatively charged, as Kuwada assumed, is quite definitely established.[37] What charge the extra-chromatin contents of the nucleus bear is not definitely known. Nearly all of the data reportedly deter-

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[35]Kuwada, Y., op. cit., p. 241.

[36]Zirkle, C., op. cit.

[37]Seifriz, W., Protoplasma, p. 354.

mining the charge of the cell components are derived from cataphoretic experiments, and, as Schrader[38] points out, the passing of a current through the cells may well cause quite abnormal conditions. The remainder of the data come from staining reactions, and unfortunately a stained cell is usually a dead cell. Although both sets of data may be questioned, it seems doubtful that both would produce the same types of abnormalities, and, therefore, the consistent results which have been obtained.

Kuwada's assumptions appear reasonable and seem to explain part of the mitotic process. However, the cause of the prophase movements of the centrosomes, of the division of the chromosomes, and of the change in the sign of the chromosome charge are left unexplained.

Koller's hypothesis. Koller[39] like Kuwada attributed the prophase and early metaphase motions of the chromosomes to attraction of the kinetochores by the equatorial area of the spindle. As an alternative to this, he suggested the repulsion between the kinetochores and the centrosomes. The metaphase equilibrium is followed by a sudden separating motion of the daughter chromosomes to a new position of equilibrium, which, in Vicia faba is about half the distance from the metaphase plate to the spindle poles. Koller attributed this motion either to the division of the kinetochores or to a decrease in the repulsion between them and the centrosomes. After a brief equilibrium in this new position, the daughter chromosomes move slowly and at an approximately uniform rate to the

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[38]Schrader, F., op. cit., p. 57.

[39]Koller, P. C., "The Movements of Chromosomes within the Cell and Their Dynamic Interpretation," Genetica, 16:447-66, 1934.

spindle poles.

Koller reported that during this motion the half-spindle between the chromosomes and the pole decreases while the interzonal fibers between the separating groups of chromosomes increase and extend. He believed this extension of the interzonal fibers to be an autonomous process and to be primarily responsible for the second part of the anaphase movement. Along with this growth of the interzonal spindle, the force of repulsion between the kinetochores and the centrosomes decreases. Since the chromosomes of one daughter group must approach each other as they converge on the pole, the mutual repulsion between their kinetochores must decrease.

In this same work, Koller reported that the arrangement of the chromosomes in the metaphase plate indicates that it is the kinetochores which reach equilibrium on the plate, not the chromosomes as a whole. The position of the bodies of the chromosomes is determined by a non-specific body repulsion acting between them. He demonstrated, by using anesthetics to prevent the formation of the spindle, that chromosome movements, both prophase and anaphase, depend on the spindle apparatus.

Through the formation of the metaphase plate this hypothesis is essentially the same as Kuwada's, and the arguments for and against the latter may be applied to it. The division of the kinetochore and the mutual repulsion of the resultant bodies might be a satisfactory explanation of the first anaphase movement if it can be shown that the time of the division corresponds to that motion. This has not been shown. The elongation of the interzonal fibers cannot be taken as a universal cause for the second anaphase motion, for, as Schrader points out, there is no

elongation of the interzonal spindle in several species.[40] Acceptance of Koller's hypothesis would therefore necessitate the assumption of more than one set of forces to explain the motions.

Darlington's hypothesis. The most recent hypothesis employing electrical forces as the basis of mitosis was offered by Darlington.[41] His assumptions were identical with those of Koller, with the exception of the explanation of the late anaphase movement. This motion Darlington contended was due to the mutual repulsion of the kinetochores coupled with a great decrease in the repulsive force exerted on them by the centrosomes. Thus he removed the principal source of objection to Koller's hypothesis. There may well be difficulty in demonstrating that the repulsion between the daughter kinetochores of the two groups is great enough to produce this motion, while the repulsion between those in each group is small enough to permit them to converge on the centrosomes.

DISCUSSION

General criticisms of electrical force hypotheses. The objections pointed out by Wilson to any bipolar force postulate have already been discussed along with the various hypotheses. These criticisms, based on the observed mutual repulsion between asters, on the existence of multipolar figures with spindles between every pair of centers, and on the crossing and anastomosing of the astral rays and spindle fibers, have

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[40]Schrader, F., op. cit., p. 46.

[41]Darlington, C. D., "The External Mechanics of the Chromosomes," Proceedings of the Royal Society of London, Series B, 121:264-319, 1936.

been shown to be invalid against any except Gallardo's first hypothesis.

Cornman[42] argued against the electrical hypotheses by noting that the spindle behaves as a structure, not as a field, since it can be moved or dissected without its parts losing their identity. This fact does not represent evidence against any of the electrical hypotheses since they all agree that once the particles of the spindle substance are oriented by the field, this arrangement persists even after the field is removed.

Many attempts have been made to influence the mitotic configuration by causing cells to divide in an electric or magnetic field. The fact that all of these have failed is interpreted by Wilson[43] as strong evidence against a major role for electric or magnetic forces. Schrader[44] states, however, that very little work has been done on the effect of strong magnetic forces, and Ssawostin[45] has shown that the rate of protoplasmic streaming in algae is definitely altered by a magnetic field. However, it may be that this is not a purely magnetic phenomenon, but an interaction of the field with the electric current set up by the streaming of the charged protoplasmic colloid particles. Hardy[46] was able to detect no effect of electric fields of 5 to 25 volts/cm. on cells in

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[42]Cornman, I., "A Summary of Evidence in Favor of the Traction Fiber in Mitosis," American Naturalist, 78:410-22, 1944.

[43]Wilson, E. B., op. cit., p. 186.

[44]Schrader, F., op. cit., p. 57.

[45]Ssawostin, P. W., "Magnetophysiologische Untersuchungen. I. Die Rotationsbewegung des Plasmas in einem konstanten magnetischen Kraftfelde," Planta, 11:683-726, 1930.

[46]Hardy, W. B., "Note on Differences in Electrical Potential within the Living Cell," Journal of Physiology, 47:108-11, 1913.

mitosis. On the other hand, using the stamen hair cells of Tradescantia reflexa, Kamiya[47] found that the spindle as a whole moved toward the anode but could not detect any relative motion between the spindle poles and the chromosomes. Such diverse results indicate the great need for more carefully controlled and executed experiments.

Experimenting with the effects of high pressures on the mitotic cycle, Pease has produced some very interesting results.[48] In general, he found that the pressure produces a decrease in the viscosity of the whole cell and tends to destroy the spindle structure. The prophase and metaphase motions of the chromosomes are completed with pressure up to 4,500 lbs/in^2, and the metaphase movements continue, though slowly, up to 10,000 lbs/in^2 pressure, even though the spindle fiber structure is destroyed at pressures of only 3,000 lbs/in^2. The metaphase and anaphase chromosomes frequently lose their hematoxylin staining capacity with pressures of 6,000 lbs/in^2 and always with 10,000 to 15,000 lbs/in^2. At pressures of 2,000 to 4,500 lbs/in^2, the anaphase daughter chromosome groups aggregate into a vesicle, which takes a shape approximately that of a tear-drop with the point directed away from the pole, and move toward the centrosomes as a group rather than individually. The chromatin appears

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[47]Kamiya, N., "Untersuchungen über die Wirkung des electrischen Stromes auf lebende Zellen. I. Das Verhalten der mitotischen Figur unter der Wirkung des Gleichstromes," Cytologia, Fujii jub. vol.:1036-42, 1937.

[48]Pease, D. C., "Hydrostatic Pressure Effects upon the Spindle Figure and Chromosome Movement. I. Experiments on the First Mitotic Division of Urechis Eggs," Journal of Morphology, 69:405-41, 1941.

_____, "Hydrostatic Pressure Effects upon the Spindle Figure and Chromosome Movement. II. Experiments on the Meiotic Divisions of Tradescantia Pollen Mother Cell," Biological Bulletin, 91:145-69, 1946.

to be scattered in islands throughout the body of the vesicle. Even when the spindle fiber structure is lacking, the chromosomes move in the region it originally occupied and do not wander out into the cytoplasm. Interzonal fibers are visible through late anaphase and sometimes even in early telophase. Pressures of 3,000 lbs/in^2 are sufficient to block the cytoplasmic cleavage, while higher pressures may even reverse a cleavage furrow which has begun.

The fact that a ten minute exposure to a pressure of 3,000 lbs/in^2 does not halt the chromosome division or movement while it does prevent the development of a cleavage furrow, even after the release of the pressure, was interpreted by Pease as showing the essential independence of the chromosome and cytoplasmic divisions.

The data show that the gel structure of the spindle is destroyed by pressures of 3,000 to 4,500 lbs/in^2, and yet the chromosomes continue to move. Since the bulk and volume of the chromosomes are large compared to those of the centriole, the resistance offered by the surrounding medium to their motion should also be comparatively large. Pease contended that under these conditions it would be logical to assume that any attractive force acting between the chromosomes and the centrioles would move the latter rather than the former. This would seem to be a logical assumption even <u>with</u> the gel structure of the spindle, for then the resistance to motion of each object would simply be increased proportionally. Since the centrioles are not moved toward the chromosomes either with or without the high pressure, it appears that they must be fixed in position by some means not affected by the pressure. Therefore, the stability of the positions of the centrioles cannot be related to the gel structure

of the spindle.

Pease states in addition that these experiments tend to discredit electric attraction or repulsion forces as the source of chromosome movement since the viscosity, and hence the resistance to motion of the chromosome path, is decreased by the pressure, while the electrical force should not be so decreased. Yet the chromosome movement is slowed by 2,000 lbs/in^2 pressure and stopped by 6,000 lbs/in^2. While it might be granted that electrical forces in homogeneous media would not be altered by these pressures, the effect of such forces in a non-homogeneous medium, such as a cell, would almost certainly be altered even if the strength of the sources remained constant. All of the hypotheses employing electrical forces to explain the mitotic movements involve particular orientations of the colloidal material between the poles and the chromosomes. For its continued existence and, therefore, for the most efficient action of these electrical forces, this orientation depends on the gel structure of the spindle area. Thus, even with sources of constant strength, the effectiveness of these electrical forces in producing motion of the charged chromosomes would be expected to decrease when the gel structure is destroyed. Furthermore, Pease has pointed out that his data show that the chromosomes frequently lose their staining capacity at pressures above 6,000 lbs/in^2 and always lose it at 10,000 to 15,000 lbs/in^2. It would seem reasonable to assume from these data that the chromosomes gradually lose that capacity as the pressure is increased. Since the staining capacity of the chromatin depends on its acidity, and that in turn determines the charge on the colloidal particles, Pease's data may be interpreted as indicating that the magnitude of the chromosome charge, one of

the sources of the electrical forces, decreases with increasing pressure. Thus, these data may prove an aid to the electrical hypotheses rather than discrediting them.

Pease also interpreted the continued motion of the chromosomes after the disappearance of the spindle structure as indicating that the spindle is not composed of contractile fibers, similar to muscle fibers, which cause the motion of the chromosomes. The shape of the chromatin vesicle, formed under pressure, also indicates that no contractile fibers are pulling it toward the centrosome. The shape of this vesicle, furthermore, may indicate that it is not being pushed toward the pole by any interzonal fibers, as has frequently been suggested. Pease further interpreted the observation that the largest and thickest of the interzonal fibers are connected to chromosomes which lag behing the others in their motion toward the poles as showing that these fibers resist the motion rather than cause it.

The interpretations based on the shape of the vesicle, however, may be quite questionable. It is doubtful if the velocity of motion of the vesicle has much to do with its shape, since this velocity is of the order of a few microns per minute even in unretarded cells. In addition, nothing apparently is known about the rigidity of this vesicle.

These experiments, according to Pease, indicate that the cause of the chromosome motion originates within the chromosomes themselves. He suggests that they cause a solation of the spindle gel on the side toward which they are moving and a regelation on the opposite side. To explain the continued chromosome movement after the visible evidence of the gel structure of the spindle disappears, Pease postulated that enough struc-

ture still exists to produce the retarded motion observed. If this is true, it appears that sufficient structure, even fibers, might remain to account for the motion on any of the other hypotheses.

According to Schrader,[49] Rashevsky concluded on the basis of some mathematical considerations, that the metaphase plate is formed through the action of inverse square repulsion forces originating at the poles. The anaphase movements result when the elastic forces exerted by the spindle fibers attached to the chromosomes exceed this repulsion and the force holding the daughter chromosomes together. Under the action of these forces, the rate of spindle elongation should show a sharp increase at the time of the chromosome separation. According to Rashevsky, the data obtained by Belar[50] on the first spermatocyte division of the grasshopper Chorthippus fit the theoretical curve very well in that they show this sudden increase. However, Schrader points out that the sudden rise shown by Belar's data occurs at an entirely different time in the motion from that required by Rashevsky's theory. Furthermore, Belar himself considers these data as only approximate since the exact position of the poles could not be determined.

In his most recent paper on this subject, Rashevsky[51] utilizes

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[49]Schrader, F., op. cit., pp. 58-60.

[50]Belar, K., "Beiträge zur Kausalanalyse der Mitose. II. Untersuchungen an den Spermatocyten von Chorthippus (Stenobothrus) lineatus Panz," Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen, 118:359-484, 1929.

[51]Rashevsky, N., "Some Remarks on the Movement of Chromosomes During Cell Division," Bulletin of Mathematical Biophysics, 3:1-3, 1941.

data given by Barber[52] on the anaphase movement in second spermocytes of Stenobothrus to show that there can be no repulsive force exerted by the poles. From calculations with these data, he concluded that both the prophase and anaphase movements are due to elastic fibers. Again the data are those of Belar, for Barber took them from the former's photographs.

The present status of the problem. The present status of the problem is clearly demonstrated by the above discussion of Pease's data. While the data are probably not to be questioned, they permit of multiple interpretation, so that almost no hypothesis concerning the forces active in mitosis can be definitely eliminated. Nearly all of the existing data can be treated in a similar manner.

The final hypothesis proposed by Gallardo cannot be accepted in its entirety since spindles with no chromatin have been shown to exist. His explanation of the mechanism of chromosome division remains as the only one suggested, though it has been completely ignored by later workers.

Lillie's hypothesis, introducing the phenomena exhibited by semipermeable membranes, was never workable. Its more reasonable features, however, have been incorporated in all of the later postulates.

The recent hypotheses of Kuwada, Koller, and Darlington have been subjected to severe criticism, but this has done no more than point out other possible interpretations. The proponents of these postulates, on the other hand, have also been unable to secure unequivocal supporting data.

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[52]Barber, H. N., "The Rate of Movement of Chromosomes on the Spindle," Chromosoma, 1:33-50, 1939.

Thus, at the present time, hypotheses based on electrical forces as the motivation of the mitotic process remain as hypotheses. A great amount of experimental evidence can be cited to support or to discredit these hypotheses, but decisive evidence is lacking.

CONCLUSION

It is apparent that the electrical hypotheses warrant further serious consideration.

The best experimental approach to the problem is not obvious. It would seem that a detailed study of the complete mitotic process in one type of cell would yield much valuable information. So far no such study has been carried out, probably because no one type shows all the steps of the process with sufficient clarity. However, since the validity of most of the hypotheses depends on the order in which the alterations of the cell and the mitotic figure occur, studies of the exact timing of these changes are sorely needed. The whole problem is, of course, complicated by the fact that no two cells respond in exactly the same manner even to the same stimuli. Therefore, it may be necessary to base the interpretation of mitotic data on statistical averages of large numbers of exact observations. Once a sound theory has been established to explain these observations, an attempt may be made to explain the individual variations presented by separate cells.

It seems obvious that additional research is needed on the effects produced by high pressures as well as those of electric and magnetic fields. In regard to the latter, it would seem very advisable to make further observations on cells in mitosis in strong electric and magnetic

fields. Observation of the cells should be continued after their removal from the field to determine whether they are still normal. If they are not, little significance could be attached to any observations made, since the abnormalities may well have produced reactions radically different from normal.

In spite of the tremendous advances made in micromanipulative technique in the past twenty years, there has been little attempt to employ this method in the study of mitosis. Micromanipulation should be an extremely valuable procedure in determining the role of electrical forces in mitosis. The range of feasible experiments in this field is almost unlimited. Some of the possibilities are outlined briefly below.

One of the more common procedures in micromanipulative studies is the injection of substances into a cell by use of micro-pipettes which are made by drawing down glass tubing to capillaries a few microns in diameter. Any magnetic forces active in mitosis should be readily demonstrated by injecting fine iron dust into the nucleus of the resting cell. If magnetic forces are operative, the dust particles should show a definite pattern in the later phases of mitosis.

It is assumed in nearly all electrical hypotheses that the centrosomal area is one of the sources of the electrical energy. Whether this source is the centrosome body itself or the centriole contained in it is not known. Micromanipulative experimentation should furnish the answer to this question. By using a micro-pipette, the centriole could be sucked out of the centrosome. By performing this operation on cells in various phases of mitosis, the effect of the loss of the centriole on the mitotic process could be determined. Obviously, if the centriole is the seat of

the mitotic force, its removal should cause extensive changes in the course of the process.

The use of micromanipulative techniques may furnish conclusive evidence of the role of electrical forces in mitosis. By filling the pipettes mentioned above with the proper electrolyte, electrodes can be formed which are not easily polarized by small currents. Two such electrodes could be introduced on opposite sides of the nucleus, and potentials applied to them. If the normal spindle formation is due to potential differences between the poles and the equatorial region, a spindle should be induced between the electrodes when the proper potentials are applied.

The objection would undoubtedly be raised that cells subjected to the experimental procedures outlined above would not be normal and that the results obtained might well be induced by the experimental procedures themselves. Until the experiments are carried out, however, it is impossible to predict the extent and significance of such effects. The micromanipulative experimentation which has been performed indicates that the injury produced in cells is not serious so long as the instruments used are small compared to the size of the cell. The careful use of micro-instruments a few microns in diameter does not seem to produce any serious injury or alteration in cells the size of an amoeba or a sea-urchin egg, which are usually 100 microns or more in diameter. Of course, the use of a micro-pipette 3 microns in diameter in a cell of 10 microns diameter might be expected to cause serious injury. With the proper selection of specimens, the use of these techniques should furnish extremely valuable data on the forces active in mitosis.

The most obvious need in further study of the role of electrical forces in mitosis is quantitative data. With the exception of the tentative beginning in this direction shown in the papers of Belar, Barber, and Ris, all on anaphase movements, there are almost no quantitative data that can be applied to this problem. Admittedly the compilation of such data will be a difficult and tedious process, for the inevitable variations in biological materials will introduce many uncontrollable factors. Nevertheless, this process must be carried out if the role of electrical forces in mitosis is ever to be determined.

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APPENDIX

A GLOSSARY OF BIOLOGICAL TERMS

Anaphase, the period of mitosis following the metaphase, during which the daughter chromosomes are moving towards the poles.

Anastral, lacking normal asters.

Aster, the morphological structure made up of the centriole, the surrounding centrosome, and the associated rays.

Astral rays, the rays or fibers extending from the centrosome or centriole.

Center, the centrosome.

Centriole, a minute, deeply staining body found in the center of the centrosome.

Centrosome, a small mass of differentiated cytoplasm forming the center of the aster.

Chromomere, one of the linear series of granules in the spireme and the chromosome.

Chromosomal fiber, a fiber connected to the kinetochore of the chromosome.

Chromosome, one of the separate, deeply staining bodies into which the substance of the nuclear network resolves itself during mitosis.

Continuous fiber, a fiber connecting the two poles of the spindle.

Cytoplasm, the substance of the cell body exclusive of the nucleus.

Equatorial plane, the plane at right angles to the spindle axis midway between the two poles.

Equatorial plate, the plate formed by the metaphase chromosomes lying in the equatorial plane.

Fibers, spindle fibers.

Interphase, the period of mitosis during which the chromatin exists as a diffuse network; the "resting" period between active divisions.

Interzonal fiber, fiber connecting separating daughter chromosomes in anaphase and telophase.

Karyomere, a vesicle surrounding a chromosome.

Kinetochore, the small segment of a chromosome to which the chromosomal fiber is attached and which seems to preceed the remainder of the chromosome in the anaphase motion.

Meiotic division, mitosis in which the number of chromosomes in the nucleus is reduced to one half the original number.

Metaphase, the period in mitosis during which the chromosomes are grouped in the equatorial plate.

Metaphase plate, equatorial plate.

Mitosis, a nuclear division which involves a spindle apparatus and the equal partition of the chromosomes to the daughter nuclei.

Monocentric, having only one center or aster.

Nucleolus, a deeply staining body found within the nucleus.

Nucleus, a body within the cell which contains chromatin.

Pellicle, sheath.

Polar granule, centriole.

Pole, the area at the end of the spindle, usually occupied by the aster.

Prophase, the period of mitosis during which the chromatin network of the nucleus changes into chromosomes.

Rays, astral rays.

Spermatocyte, a cell from which the sperm cells are derived.

Spindle, the configuration of the cell substance between two asters.

Spindle fiber, a longitudinal striation of the spindle which has the appearance of a fiber.

Spireme, the chromatin threads found in the prophase nucleus.

Subterminal, closer to the end than to the middle.

Telophase, the period of mitosis following anaphase during which the chromatin is reformed into the network of the interphase nucleus.